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Juvenile Myelomonocytic Leukemia (JMML) With the Hematologic Phenotype of Severe β Thalassemia

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A 3-year-old Filipino-American child with recurrent fever, splenomegaly, anemia, and thrombocytopenia, was found to have a hemoglobin F level of 76.9%. His reticulocyte count was elevated (4.3%), and erythroblasts were present in his peripheral blood. The child's erythrocytes were microcytic (MCV 66.9 fl) but his serum ferritin level was normal. His bone marrow at initial presentation demonstrated normal cellularity without an increase in blast cells. The disease progressed with worsening anemia, leukocytosis, and thrombocytopenia, with increased blasts in his marrow and the appearance of a mediastinal mass. His liver, spleen, and lymph nodes were found to be infiltrated with myeloblasts, supporting a diagnosis of juvenile myelomonocytic leukemia (JMML). Analysis of the child's Hb F showed a $^{G}\gamma/^{A}\gamma$ ratio of 2.2, which was within the characteristic range for JMML. A globin synthesis study using blood reticulocytes showed an α/non-α globin synthesis ratio of 2.24, typical of severe homozygous β thalassemia. Southern blot analysis of blood-leukocyte DNA from the patient and his parents demonstrated no apparent abnormality in the β-globin gene promoter or coding regions. The elevated level of Hb F in this child with JMML appeared to be part of an acquired Cooley's anemia-like hematologic phenotype. Am. J. Hematol. 58:67–71, 1998. © 1998 Wiley-Liss, Inc.

Key words: juvenile myelomonocytic leukemia; fetal hemoglobin; hemoglobin synthesis

INTRODUCTION

Juvenile myelomonocytic leukemia (JMML), previously referred to as juvenile chronic myeloid leukemia, is an uncommon disease of infants and young children. The disorder is expressed as a clonal panmyelopathy [1–3] that is distinct from Philadelphia chromosome-positive adult CML. Children with type I neurofibromatosis account for 7% of the reported cases of JMML [1,4]. Approximately 30% of JMML patients have been shown to have mutations of the N-*ras* proto-oncogene [5].

Typical clinical features of JMML include leukocytosis with the presence of early myeloid and monocytic elements, thrombocytopenia, skin rash, and hepatosplenomegaly. Progression of the disease is often rapid, with infiltration of the bone marrow and other tissues with monocytic and myeloid cells.

A remarkable finding in a majority of children with the JMML syndrome is the presence of strikingly elevated levels of fetal hemoglobin (Hb F) [6]. The $^{G}\gamma/^{A}\gamma$ ratio of the Hb F from these patients has been found to be similar to that of Hb F from normal newborn infants [7–9], which, taken together with their characteristically low levels of carbonic anhydrase and high i antigen titers, has suggested that erythropoiesis in JMML may reflect a reversion to a fetal pattern. Studies of Hb F synthesis by BFU-E-derived bursts [8] have provided further support for this notion.

In this report, we describe the findings from a child with the JMML syndrome with greatly elevated levels of Hb F, whose pattern of hemoglobin synthesis suggested the presence of an acquired abnormality with β thalassemia-like hematologic features.

METHODS

Hemoglobin Studies

Procedures for the preparation of hemoglobins for analysis, Hb electrophoresis, and measurements of Hb F

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TABLE I. Representative Hematologic Values From the Patient and His Parents

	Patient	Mother	Father
Hemoglobin (G/dl)	10.5	14.8	14.9
MCV (fl)	67	89	95
Reticulocytes (%)	4.3	0.5	0.8
Platelets ($\times 10^{-3}$)	32	255	214
Hemoglobin electrophoresis	FA	AA	AA
Hb A_2 (%)	0.4	2.6	3.3
Hb F (%)	76.9	0.3	0.3

and Hb A_2 were as described previously [10]. For the quantitative estimation of globin chains, HPLC was performed with a C_4 column (Vydac, The Separations Group, Hesperia, CA) with acetonitrile-water-trifluoroacetic acid gradient elution [11]. For globin synthesis studies, washed red cells were incubated for 2 hr at 37°C in medium containing L-leucine-^{3}H; radiolabeled globin chains were fractionated by carboxymethylcellulose column chromatography [12].

DNA Analyses

High-molecular-weight DNA was prepared by phenol/chloroform extraction [13] from blood leukocytes and from formalin-fixed splenic tissue. The procedure for identification of N-*ras* gene mutations was as previously described [14].

For Southern blot analyses, DNA from normal controls and from the patient and his parents was digested with Bam HI, Hind III, Eco RI, or Pst I, and hybridized with a full-length human cDNA β globin-gene probe [13,15,16]. In studies that examined the 5′ β globin-gene promoter region, the segment between nucleotides 40250 and 41270 [17] was amplified by PCR, followed by digestion with Bam HI.

CLINICAL FINDINGS

A previously well 3-year-old boy of Filipino parentage was evaluated because of recurrent fevers and lethargy. His liver was found to extend 1.5 cm below his costal margin, and his spleen 4 cm. No apparent rash or other abnormality was found from his examination.

His hematologic findings were: WBC 9,600/μl; Hb 10.5 G/dl; platelets 32,000/μl; RBC 4.07×10^6/μl; MCV 66.9 fl; and reticulocytes 4.3%. His blood counts also showed variable numbers of erythroblasts. His serum ferritin level was 78.2 μg/ml. His bone marrow had normal cellularity with erythroid hyperplasia, but no dyserythropoietic changes or increase in blast cells. Cytogenetic analysis revealed a normal 46XY karyotype. Hemoglobin studies demonstrated a greatly elevated level of Hb F (Table I). Hematologic values from both of the parents were normal.

Over the following month, the child's spleen became progressively larger accompanied by abdominal pain. His leukocyte count increased to 60,000/μl, and his platelet count decreased to approximately 20,000. Examination of his bone marrow revealed an increase in blast cells to 7%. Computed tomography of his chest and abdomen demonstrated the presence of a mass bordering the right lower mediastinum, as well as periaortic lymph node enlargement. Biopsy specimens of the child's spleen, liver, and lymph nodes showed infiltration with myeloblasts, supporting a diagnosis of JMML.

A chemotherapy regimen was initiated that included dexamethasone, etoposide, daunorubicin, cytarabine, thioguanine, and 2-chlorodeoxyadenosine. The child showed no appreciable response to this treatment, and he subsequently underwent a haploidentical transplant with bone marrow from his mother. Engraftment was successfully achieved, with normalization of his hematologic values, but 5 months later he experienced a recurrence of his disease, which progressed with a rapidly downhill course.

RESULTS

Hemoglobin Findings

Electrophoresis of the patient's hemoglobin demonstrated an FA pattern with a low percentage of Hb A_2. Smears of his peripheral blood prepared according to the procedure of Kleihauer et al. [18] demonstrated strongly-positive staining of Hb F in all of the red cells. These studies from both of the parents yielded entirely normal results.

HPLC fractionation of the child's globin chains (Fig. 1) confirmed that the non-α chains were predominantly of the $^G\gamma$ type, with a $^G\gamma/^A\gamma$ ratio of 2.2.

Globin Synthesis Results

Blood reticulocytes from the patient actively incorporated radiolabeled L-leucine into globin chains. The relative rates of synthesis of the individual globin types are shown in the chromatogram in Figure 2. The synthesis of γ chains accounted for approximately 65% of the non-α chains, in close correspondence with the percentage of Hb F in the child's blood. As the chromatogram in Figure 2 also demonstrates, α chain synthesis was substantially in excess of non-α chains, with an $\alpha/[\beta + \gamma]$ chain synthesis ratio of 2.24. Globin synthesis studies using reticulocytes from the parents yielded low levels of radioleucine incorporation into globin chains; the α/non-α globin synthesis ratios from both of them were within the range of normal.

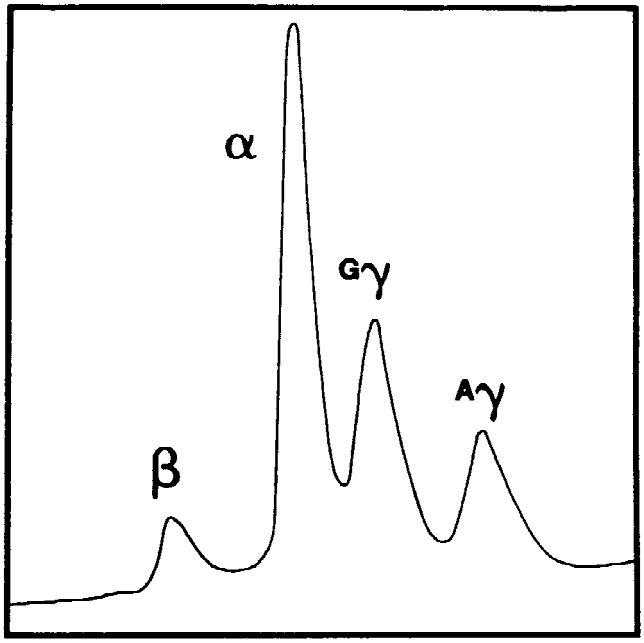

Fig. 1. HPLC fractionation showing the representation of globin chains in the patient's hemoglobin.

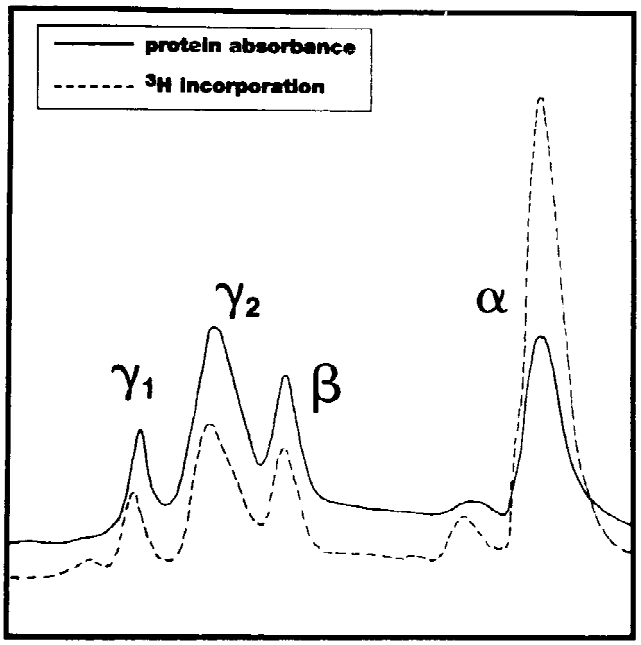

Fig. 2. Incorporation of L-leucine-^{3}H into globin chains by reticulocytes from the patient's blood.

DNA Studies

DNA from the patient's blood leukocytes and splenic tissue showed no evidence of mutation in the N-*ras* gene. Southern blot analyses of the β globin gene coding region and upstream promoter region, using DNA from the patient and his parents, yielded restriction enzyme fragments that invariably were indistinguishable from those of normal controls (data not shown).

HLA/DR Studies

Histocompatibility testing of the patient showed him to be: A11, A34, B40, B62, DR 12, DR 15. Results from his mother were: A24, A34, B35, B40, DR 12, DR 15; and from his father: A11, A34, B62, DR4, DR 12.

DISCUSSION

The nonspecific clinical findings early in the course of this child's illness made it difficult initially to arrive at a specific diagnosis. As his disease progressed, with the appearance of blast cells in his bone marrow and in other tissues, and in the face of his characteristic pattern of Hb F expression, it became apparent that this syndrome represented JMML. Although elevated levels of Hb F as an acquired abnormality have been identified in patients with a variety of forms of leukemia, Hb F levels in excess of 50% appear to be unique to the JMML syndrome [19].

The increased $^{G}\gamma/^{A}\gamma$ ratio of this patient's Hb F, together with his very low level of Hb A_2, were entirely comparable to findings from other children with JMML [7,8,9,19]. These specific changes, however, in addition to occurring in the fetus and newborn, have also been observed in patients with severe forms of β thalassemia from a wide variety of ethnic backgrounds [20–24].

Our findings from the globin synthesis study of this patient (shown in Fig. 2) included a highly unbalanced synthesis pattern, with a substantial excess of α chain synthesis in relation to that of the non-α chains. These results were indistinguishable from those obtained in similar studies of patients with severe β thalassemia [25,26].

This child's clinical syndrome also included many of the other characteristic hematologic features of severe β thalassemia: His blood erythrocytes were microcytic and dysmorphic, and his reticulocyte count was consistently elevated and accompanied by a significant degree of normoblastemia.

The other hematologic data from the patient and his parents provided no indication of a genetic basis for his Cooley's anemia-like syndrome: Both parents had normal hemoglobin levels as well as normocytic red cell indices, their levels of Hb A_2 and Hb F were not elevated, their erythrocytes showed no evidence of Hb F-containing cells by the Kleihauer-Betke procedure, and the patterns of globin synthesis by reticulocytes from their blood were also within the normal range. Southern blot analysis of the β-globin gene coding region and upstream promoter region from the patient's DNA also produced patterns that were indistinguishable from those of normal controls.

We are not aware of other studies of globin synthesis from patients with JMML, and it may be possible that the elevated levels of Hb F in others of these patients also represent part of an acquired β thalassemia-like syndrome.

Hematologic disorders having many features in common with α thalassemia, expressed with the phenotype of Hb H disease, have been observed as acquired abnormalities in patients with a wide variety of other blood diseases, including acute lymphoblastic leukemia [27], erythroleukemia [28,29], adult CML [30], sideroblastic anemia [31,32], myelofibrosis [33,34], and other forms of myeloproliferative disease [35,36].

Efforts to identify the underlying molecular abnormality in these acquired Hb H disease syndromes have been only partly successful. Studies of several of these patients [32,36,37] have shown markedly decreased levels of α-chain mRNA in their hemoglobin-synthesizing cells. However restriction enzyme mapping studies of their α-globin and ζ-globin genes were unrevealing of any abnormality [36,37].

In an effort to determine if the decreased α-globin gene expression in these patients was directly related to the α-globin genes on chromosome 16, Helder and Deisseroth [38] performed experiments in which the chromosome 16 from several affected individuals was transferred to mouse erythroleukemia cells. S1 nuclease analysis showed that in a number of such mouse cell lines, human α-globin gene expression was similar to that of cells containing human chromosome 16 obtained from normal individuals. These observations suggested, therefore, that the suppression of α-globin synthesis in these patients with the acquired Hb H syndrome might well be the result of a genetic abnormality involving a factor acting in trans to the α-globin gene.

Acquired forms of microcytic anemia with β thalassemia-like expression have been described only rarely. In reports of two such syndromes [39,40], one involving an adult with erythroleukemia and the other an elderly individual with refractory anemia accompanied by ring sideroblasts, both of the patients were described as having high levels of Hb F with lower-than-normal levels of Hb A_2; In light of these findings, both of these acquired syndromes were characterized as being δβ thalassemia-like.

Globin synthesis studies of additional patients with JMML will be needed to test our hypothesis that the elevated level of Hb F associated with this disease may be one element of an acquired β thalassemia-like syndrome. If this indeed proves to be the case, JMML patients could provide a further opportunity to gain an understanding of the underlying molecular basis of these acquired abnormalities.

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